The Effect of High Levels of Certain Steroids on Induced Lymphocytic Leukemia in the Rat

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If one accepts the established fact that the adrenal steroids have regulatory powers over normal lymphoid tissue, it would seem reasonable to assume that the adrenal steroids may also regulate the immature cells of lymphocytic leukemia.

Since the advent of the clinical use of cortisone and of ACTH in April, 1949, many studies on both animals and human beings have been attempted to determine whether cortisone or the adrenocorticotropic hormone had any effect on the lymphoid cells of neoplastic origin. The results reported in the literature are widely diversified. In some cases of acute leukemia definite improvement, from a hematologic standpoint, was reported. Marrows have been said to revert to a normal picture, and the peripheral blood smears revealed no suggestion of cellular immaturity. These cases, however, have been exceptions. Not only have the great majority of acute leukemias failed to respond, but, at times, the course of the disease has actually appeared to be accelerated by treatment with either cortisone or adrenocorticotropic hormone. Those few remissions that have been reported to occur were never permanent, and, sooner or later, the blood picture changed from the normal back to the characteristic malignant pattern of acute leukemia. Subsequent courses of therapy usually failed to produce any further remissions.

As yet, there has been no satisfactory explanation of the action of the adrenal cortical hormones on the blood elements. However, numerous investigators have concluded that lymphoid tissue, both fixed and circulating, is probably an end organ over which certain steroid hormones of the adrenal cortex have the power of regulation. Evidence for this concept can be found in the earlier medical literature. For example, as early as 1895, Star (10) reported the case of a young girl who had died in an Addisonian crisis; at necropsy, marked enlargement of the thymus and other lymphoid tissues was observed. It was 2 years later that Bardeen (1) reported his observations on patients who had died following extensive superficial burns. These individuals displayed an atrophy or a diminution of the lymphoid tissue. Thus, we might project these two independent observations to our present-day understanding. The low level of adrenal steroids, in Addison's disease, permits or fails to inhibit lymphoid hyperplasia. On the other hand, high levels of circulating adrenal steroids, which are present during the alarm reaction or long periods of stress, are capable of inhibiting normal lymphoid growth. At times involutionary changes will become apparent. The latter situation is analogous to those cases of extensive superficial burns described by Bardeen, when stress was produced, stimulating the release of high levels of steroids into the blood stream.

On the basis of the foregoing evidence it is logical to approach the problem of therapy of leukemia by the administration of adrenal cortical steroids. If the leukemic cell of lymphoid origin has basically the same chemical structure as the immature cell of normal lymphatic tissue, it is conceivable that high levels of adrenal steroid may also inhibit the proliferation of these abnormal cells. Previous studies have failed to establish clearly the hormonal control of these leukemic cells. This suggests either that the leukemic cell does not have the basic physicochemical structure present in the normal cell or that the action of the steroids is blocked by some unknown factor or factors.

Heilman and Kendall (3) induced a regression in the size of growing transplanted lymphosarcomas in mice by the administration of Compound E. While the tumors did show marked regression during the period of therapy, discontinuance of the hormone resulted in regrowth of the tumors with ultimate death of the animal. They concluded that "the influence of Compound E appears to depend upon stimulation of the rate of the catabolism of proteins to a degree which resulted in the death of malignant cells. The normal tissue
cells of the animal, although they might be placed under severe strain, did not appear to undergo permanent injury.”

Prior to the publication of these data, Murphy and Sturm (9) had shown that adrenalectomy significantly reduced the natural resistance of rats to a transplantable lymphatic leukemia. Their percentage of successful transfers was 89.7 per cent in adrenalectomized animals, as compared to 48.5 per cent in a control group of normal animals. Later they showed that an increased resistance to transplantable lymphoid tumors in rats was induced by the administration of adrenal cortical extracts in oil.

Law and Spiers (5) noted a decrease in the total immature lymphocytes in mice with spontaneous lymphatic leukemia after the injection of adrenal cortical extract. Examination of the tissues of these animals revealed a regression in the infiltration of the thymuses and lymph nodes of those animals that received adrenal cortex, as compared to the untreated controls. There was no apparent difference in the mortality rates of the two groups, although the treated group survived for longer periods after the development of leukemia than the controls.

Levin (6) attempted to evaluate the role of the adrenal cortex in lymphoid leukemia. He did this by determining the amount of cholesterol in the adrenal glands of mice before and after the development of the leukemic state. Although the weight of the adrenal glands was increased by 58.0 per cent following the development of leukemia, the amount of cholesterol in these glands was markedly reduced. If we analyze these findings, we can readily see that the leukemic state produced changes in the adrenal gland analogous to the stress produced by lethal doses of toxins, infectious diseases, burns, hemorrhage, and traumatic shock. Thus, there is apparently a nonspecific relationship between the adrenal cortex and the leukemic state, even though the cells of lymphoid origin act as an end organ to the influence exerted by the adrenal cortical steroids.

In attempting to determine the effect of adrenal stimulation on leukemia, Levin administered pituitary adrenocorticotropic hormone from the time of transmission of the leukemia until the time of death in a small series of mice. He was not able to demonstrate any significant difference in the course of the disease in the treated group as compared to the untreated control leukemic animals.

These results with adrenocorticotropic hormone were not in agreement, however, with those of Murphy and Sturm (9). They had shown that ACTH prevented the development of leukemia in 75 per cent of one series of rats, while the control series of animals had a mortality rate of 90 per cent.

Lewis, Aptekman, and King (7) reported a retarding action of the adrenal gland on the growth of sarcoma grafts in rats. By mincing adrenal tissue and implanting it with the tumor fragments they were able to prevent the growth of the tumor in the recipient animal. But if minced kidney, spleen, liver, or thymus tissue were transplanted with the sarcoma, tumor growth was even more rapid than it was in the control group of animals in which the sarcoma had been implanted alone.

Higgins and Woods (4) showed that cortisone did restrict the leukemic process in mice, and they were able to extend the survival time from a mean of 8.7 days to one of 11.9 days. Burchenal, Stock, and Rhoads (8) reported the effects of the administration of both cortisone and ACTH to mice into which AK4 mouse leukemia had previously been transplanted. They showed that cortisone, and to a lesser degree ACTH, produced an anti-leukemic effect on the normal progress of the disease. This was demonstrated by the absence of, or minimal, leukemic infiltration into the liver of those mice that received cortisone, as compared to the heavy infiltration of cells of leukemic origin into the parenchyma of the control animals. The survival time of the treated animals was not significantly altered from that of the untreated control mice.

METHODS

Rats of the Sprague-Dawley strain, weighing between 60 and 90 gm. and ranging in age from 4 to 6 weeks, were selected for our study. All animals were housed in metal cages, kept on wire screen, and were provided with an adequate commercial ration.

The lymphocytic leukemia was that which had been developed at the Rockefeller Institute by Murphy and Sturm (9). They had produced lymphosarcoma in a Wistar rat by injecting 1,2,5,6-dibenzanthracene subcutaneously into the groin. This tumor was found to be easily transplantable. Unique among its characteristics was its ability to have a dual growth pattern. The subcutaneous injection of a cellular suspension of this tumor produced a rapidly growing lymphosarcoma that killed the rat in a period of from 18 to 22 days. When this material was injected into the peritoneal cavity, an acute fulminating lymphocytic leukemia occurred which produced death in from 7 to 10 days.

At all times an adequate stock of rats with lymphosarcoma was kept on hand, from which donor tissue could be obtained. These lymphosarcomas
grew to be very large, often attaining a size of 7×4 cm. by the third week. They rarely metastasized to distant parts of the body, but they invaded the neighboring tissues by direct extension. Despite the large size of these tumors, central necrosis rarely occurred. This was most unusual, since there is a relative avascularity, as would be expected in any tumor with so fast a growth. The tumor is unusually firm, and the cut surface is glistening and white. The lymphosarcoma is particularly suitable for transplanting to large numbers of animals, since most of the cells are viable and no tissue has to be discarded. Histologically, the tumor is composed of small round cells, which are uniform in size. Scattered throughout are larger cells with definite reticular characteristics. In the young tumor, many mitotic figures may be seen. The stroma is scant in the small tumors but increases in amount with the age and the size of the lymphosarcoma.

We have found that it is possible to control the degree of malignancy by varying the age of the donor cells. Thus, we can increase the malignancy and lower the life expectancy in the second generation by allowing the donor tissue to achieve a maximal period of growth. Conversely, the life expectancy can be prolonged in the second generation by utilizing the donor tissue after a short period of growth.

Transfers were made of donor tissue, obtained by a sterile method, from animals into which tumor tissue had been implanted 9–11 days previously. A cellular suspension was prepared in the following manner: the lymphosarcoma was removed from donor animals immediately after they had been killed by etherization. Sterile iridectomy scissors and small thumb forceps were used in cutting the tumor into small fragments, which were then macerated in a sterile crucible. A double volume of sterile isotonic saline solution was then added to the tissue, and the resultant mixture was strained through four layers of gauze. One-half cc. of this cellular suspension was injected into the peritoneal cavity of each animal.

Blood samples were obtained from tail veins, and total leukocyte and differential distributions were determined in the usual way. Adrenocorticotropic hormone and cortisone were given intramuscularly every 12 hours during test periods.

RESULTS

Induced leukemia in the normal animal.—Six series of animals, with a total of 240 rats, were used in evaluating the total leukemic picture in this strain of rats. All these animals were approximately the same size and weight. The percentage of successful transplants resulting in a fulminating leukemia in the six series of normal animals ranged from 84 to 100 per cent, with an over-all take of 88 per cent. That is, 211 of the 240 animals inoculated with 0.5 cc. of the cellular suspension died with acute lymphocytic leukemia. The blood of 60 of these leukemic animals was examined daily to appraise the changes in the total and in the differential leukocyte counts. Not all the animals developed leukocytosis, for some of them developed marked leukopenia.

Necropsy was performed on all 60 animals, and sections of the adrenals, thymus, spleen, liver, and lymph nodes were prepared (Figs. 1 and 2). In every animal leukemic infiltration into the thymus, the lymph nodes, mesentery, liver, spleen, and bone marrow was noted. The most striking changes were observed in the thymus, where the leukemic infiltration produced as much as a 400 per cent increase in size. The leukemic infiltration into the marrow completely overshadowed the myelopoiesis or erythropoiesis which is normally present. The leukemic cell infiltration into the liver was concentrated mainly around the portal vessels at the periphery of the lobules, but there was some dissemination around adjacent sinusoids. There was no apparent infiltration into the adrenal glands, and yet the weights of these organs were considerably increased when compared to the weights of adrenals of those few animals in which leukemia had failed to develop (Chart 1).

The effect of ACTH in the normal animal.—Ten normal, male rats, varying in weight between 65 and 80 gm., were given intramuscularly 1 mg. of ACTH per rat every 12 hours for a period of 10 days. These animals were examined every third day to determine changes in body weight as well as changes in the total and the differential leukocyte counts. On the eleventh day, necropsy was performed on five of the ten animals, and five were allowed to live for 5 days longer.

A decrease in the total number of leukocytes was observed in each animal that had received ACTH, with a relative and absolute decrease in the number of lymphocytes and a relative and absolute increase in the number of neutrophilic leukocytes. After the withdrawal of ACTH, there was some tendency for the number of lymphocytes to return to normal, but the initial level had not been attained at the time the animals were killed on the sixteenth day. The total number of neutrophils quickly dropped to the initial level 2 days after ACTH was discontinued.

In animals, killed on the eleventh day, that had received ACTH for the preceding 10 consecutive days, a significant increase in the size of the adre-
nal glands and a marked decrease in the size of the thymuses were noted when compared to the weights of these organs in untreated controls of the same age and body weight.

Discontinuance of the ACTH for 5 days resulted in a significant gain in body weight. The thymus showed some tendency to regenerate during the 5-day recovery period, and the adrenals were smaller than before, approaching the size of the adrenals of the untreated controls.

The effect of cortisone in the normal animal.— Cortisone was administered intramuscularly to 10 rats for 10 days in amounts of 1 mg. every 12 hours. Five animals were killed on the eleventh day, and the remaining animals were killed on the sixteenth day, 6 days after cortisone was discontinued.

The total leukocyte count in all rats receiving cortisone dropped to a much lower level with a greater relative and absolute decrease in the lymphocytes than occurred in animals receiving ACTH. The relative and absolute neutrophilia was also more marked in rats on the cortisone regimen than in those given ACTH.

The animals appeared lethargic on the eighth day of the administration of cortisone. The lethargy, however, was not progressive, and their activities were only moderately less than normal on the last day of the administration of the hormone. Loss of body weight was about the same in each animal during this 10-day period. Discontinuance of the cortisone resulted in a significant gain in weight in the five rats that were allowed to recover from the toxic effects of such large doses of hormone. There was marked thymic atrophy in animals receiving cortisone, and their adrenals were atrophic, as was anticipated.

On withdrawal of cortisone from five of the animals on the tenth day, a prompt improvement in activity and an increase in body weight were recorded. At necropsy, 6 days later, the thymus and the adrenals were significantly larger than those observed in animals killed after 10 days of cortisone therapy.

The effect of ACTH in animals bearing a transplanted lymphatic leukemia.—Forty rats of approximately the same size were inoculated with leukemia in the manner described previously. On the fourth day after inoculation of the leukemic cells, ACTH therapy was instituted on 20 of these 40 animals in amounts of 1 mg. every 12 hours. Intramuscular injections were continued for 10 days to all animals that survived for this entire period of treatment. The approximate time of death was recorded for each animal comprising both the test and the control groups.

The results of this initial study with adrenocorticotropic hormone indicated that its administration to rats bearing leukemia had ameliorated the disease process somewhat. There was an increase in the survival time of the animals that received ACTH; but the difference in the mortality rate in the two groups was so slight as to be statistically insignificant, for a 20 per cent survival was recorded for the group which received ACTH, while 10 per cent of the untreated control group survived (Chart 2).

Since leukemia in these animals had developed for 4 days and was certainly fulminating, it was thought that the proliferation of leukemic cells had reached such a magnitude at the time ACTH was first given as to make the treatment relatively ineffective.

Accordingly, a second series of 45 animals of the same size and age was selected to constitute a second test group. Twenty-five of these served as controls, and 20 were inoculated with leukemic cells. On the day of inoculation and daily thereafter these 20 rats were given 1.0 mg. of ACTH

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<th>Leukemia, no Rx</th>
<th>Leukemia, ACTH</th>
<th>Leukemia, cortisone</th>
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**CHART 1.**—Organ weights of animals with leukemia revealing deviation from normal, as well as changes provoked by ACTH and cortisone therapy. All weights are expressed in milligrams per hundred grams of body weight. Number of animals on which necropsy was performed is given in circles.

**CHART 2.**—Viability curve. ACTH begun after leukemia had been established.

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every 12 hours for a period of 12 days. The blood of each animal in the hormone-treated group was studied on the first, fourth, and eighth day of therapy to determine the effect of ACTH on the leukocyte distribution.

There was no appreciable change in the total leukocyte count of the treated animals when they were compared to the animals that had received no treatment. But there was a marked neutrophilia, with an average of about 20,000 neutrophils per cubic millimeter of blood, recorded on the eighth day for animals given ACTH. The number of lymphocytes, both mature and immature, was considerably reduced, but again there was no appreciable difference in the mortality rates of the treated and the untreated groups. Survival time was only slightly prolonged: those leukemic animals receiving ACTH from the day they were inoculated lived, on the average, only 24 hours longer than the untreated controls (Chart 3).

Weight changes of the organs in the ACTH-treated group varied but slightly from those in the control group (Chart 1). Decreases in the weights of the spleen and thymus were due primarily to the lysis of normal lymphoid tissue induced by the hormone, and the marked increase in weight of the adrenal glands was, of course, due to its corticotrophic effect. A slight decrease in the weight of the liver represented, we believe, an actual retardation of the leukemic infiltration into this organ.

Histologic examination of certain tissues showed marked changes produced by ACTH. The malignant invasive pattern, however, characteristic of control leukemic animals was maintained in many organs of those receiving ACTH; but there were alterations in the cells of leukemic origin. There were marked degenerative changes in these infiltrating cells, consisting of a decrease in their size and a marked vacuolization of their cytoplasm. In all lymphoid tissues throughout the organism a lympholysis of all normal cells of lymphoid origin had occurred. Normal lymphocytes were not seen in any of the sections of the thymus that we examined (Fig. 2b). There was a definite decrease in the extent of leukemic infiltration into both the liver (Fig. 16) and the spleen of the ACTH-treated animals. In a few test animals the leukemic infiltration was minimal, but in every instance there was some involvement of the pertinent organs. The bone marrow in ACTH-treated animals showed little if any change from the pattern of extensive leukemic involvement seen in the marrows of the control leukemic animals.

The effect of cortisone on transplanted lymphatic leukemia.—Twenty animals of the same sex and age as used in the ACTH study were selected and inoculated with leukemic cell suspension. One mg. of cortisone was administered every 12 hours for a period of 12 days to all animals that survived for this period of time. Cortisone was given on the day of the transplant and on each succeeding survival day for 12 days.

Blood of these animals was examined on the first, fourth, and eighth day following inoculation for the changes in the total and the differential leukocyte distribution. Counts taken after the eighth day were considered statistically unreliable, as the viability rate decreased precipitously beyond this time, making it virtually impossible to record accurate data on so small a number of animals.

The administration of cortisone produced a rather marked leukocyte response in these leukemic animals. There was a marked lymphopenia, followed by a relative and an absolute increase in the number of neutrophils. The total number of neutrophils in these leukemic animals, however, never attained so high a level as it did in normal animals given the same amounts of cortisone. This was considered caused by the fact that in leukemic animals most of the myelopoietic centers had been reduced or destroyed by the leukemic infiltration.

The average weight of the thymus in leukemic animals receiving cortisone was less than one-sixth that of the thymus in leukemic animals which did not receive the hormone (Chart 1). Histologically, the thymus consisted essentially of circling strands of loose stroma, separated by a few undifferentiated cells (Fig. 2c). True lymphocytes were not encountered in the thymus nor in any other organ. Approximately the same destructive effect of these amounts of cortisone was observed in the spleen as in the thymus, but to a lesser degree. Leukemic infiltration into the liver had not occurred, and the perivascular spaces were entirely free of leukemic cells; only normal parenchymal cells of the liver were present (Fig. 1c).

An interesting sidelight to this study was noted
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on examination of histologic sections of the pituitary gland. So-called Crooke's changes, which consist of a hyalinization and a vacuolization of the basophil cells, were present in all the pituitary glands of animals which had been given cortisone. These changes in the basophils, which are believed to be induced by high levels of circulating steroids, certainly gave evidence of the intensity of the therapy we used in these leukemic animals.

Despite the apparent lytic effect of cortisone on the leukemic infiltration in the liver and the histology of the spleen and thymus, certain differences in the mortality rates of control and test groups of animals did not occur (Chart 4). Again, however, and to a much greater degree than in leukemic animals receiving ACTH, the survival time of these animals receiving cortisone was prolonged. The cortisone-treated animals lived for an average of 12 days, as compared to an average of 9 days for the untreated leukemic animals.

CONCLUSIONS
A study designed to appraise the influence exerted by either adrenocorticotropic hormone or cortisone on the course of a transplanted lymphatic leukemia in rats has been reported. The data appear to warrant the following conclusions:

1. High levels of either ACTH or of cortisone—1 mg. every 12 hours during the survival period, following inoculation—did exert a suppressing influence on the neoplastic lymphoid tissue. These hormones did not prevent the development of the leukemia, following intraperitoneal inoculation with leukemic cells, but each appeared to ameliorate the disease process somewhat and to prolong the survival time slightly.

2. High levels of these hormones definitely altered the histologic structure of the thymus, the spleen, and other lymphoid organs. Leukemic infiltration into the liver was definitely restricted by the administration of these hormones.

3. Leukemia, thus induced, exerts a severe stress phenomenon within the animal, resulting in marked hypertrophy of the adrenal cortices and an elevation of the absolute numbers of neutrophilic leukocytes in the peripheral blood.

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